What can the different current-detection methods offer for element speciation?

Jörg Feldmann

Elemental mass spectrometry (MS) has revolutionized the field of element speciation in the past decade. Since the late 1990s, molecular MS has increasingly become more popular. Although the potential of the latter technique has been recognized by the element-speciation community, it has not yet gained general acceptance. Only recently, the tool-box of the analyst interested in element speciation has expanded dramatically due to the technical developments in molecular MS and in X-ray absorption spectroscopy (XAS). In particular, the development of more user-friendly ionization methods, such as atmospheric pressure chemical ionization (APCI), electrospray (ES), matrix-assisted laser desorption/ionization (MALDI) and, most recently, atmospheric pressure photoionization (APPI), as well as the installation of ultra-dilute XAS stations, has made it possible for these methods to be applied for element-speciation analysis of heterogeneous real samples. These and other emerging techniques will be discussed, especially in the light of the new challenges in element speciation, i.e. the detection of unstable short-lived species and macromolecules. This review attempts to show the advantages and the limitations of the various techniques, their combinations in off-line set-ups and on-line dual-detection modes for the analysis of mainly biological and environmental samples.

Keywords: Atmospheric pressure chemical ionization; Atmospheric pressure photoionization; Electrophoresis; Electrospray; Element speciation; Gas chromatography; Liquid chromatography; MALDI; Mass spectrometry; X-ray absorption spectroscopy

1. Introduction

This review will discuss how powerful different analytical techniques are in terms of their detection capabilities for various types of element species and the quality of information gained about the element species. The most powerful speciation methods are listed in Table 1; most of them are combined techniques, i.e. on-line sample-clean-up or species-separation techniques with powerful molecular and/or elemental detectors. I give an overview of recent developments in traditional speciation methods and studies that have benefited from newly emerging methods.

2. Elemental MS

Atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS) have been used for speciation analysis, but AAS is not sensitive enough and AFS has limited applicability (see Table 1), so the focus will be on inductively coupled plasma MS (ICP-MS) as a versatile detector for elemental speciation analysis.

ICP-MS can be used for molecule identification only when combined on-line or off-line with a molecule-specific separation technique, because the ionization of the molecules does not retain any molecular information. Liquid chromatography (LC) and gas chromatography (GC) as well as electrophoretic methods, such as capillary electrophoresis (CE) and recently gel electrophoresis (GE), have been combined extensively with ICP-MS for the determination of different elemental species.

The real strength of ICP-MS is that the molecular occurrence of the element of interest does not influence the response significantly and that the technique shows only limited matrix influence, which results in a species-independent response. Hence, stable elemental species can be used for quantification purposes for a series of different species of one element [1]. This becomes very useful when the analyte is an unstable species, an unknown elemental species or a species of which no pure standard is available.

The limits of detection (LODs) for different species are slightly different due to the varied peak width of the eluting species. Castelhouse et al. give an example [2], in which different arsenic species in fertilizers have been determined. The peak...
Table 1. Combined and non-combined techniques, their detection capabilities, major advantages and limitations

<table>
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<tr>
<th>Molecular specificity</th>
<th>Element selectivity</th>
<th>LODs</th>
<th>Molecular information</th>
<th>Matrix and matrix effects</th>
<th>In situ methods</th>
<th>Major advantages</th>
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<th>Major application</th>
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<tr>
<td>GC</td>
<td>AFS</td>
<td>μg/L range</td>
<td>Only RT comparison with standards</td>
<td>Volatile organics, water</td>
<td>NL, (s), (D)</td>
<td>Sensitive for volatile Hg, As</td>
<td>Only for volatile Hg in gas. Methylmercury with HG As, Sb, Se speciation in liquid samples</td>
<td></td>
</tr>
<tr>
<td>HPLC-HG</td>
<td>AFS</td>
<td>μg/L range only for HG active elements</td>
<td>Only RT comparison with standards and HG behavior</td>
<td>Redox active metals</td>
<td>NL, D</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HPLC</td>
<td>ICP-MS (quadrupole)</td>
<td>Isotope-specific response As: (1 μg/L); Se: m/z 82, 77; (5 μg/L); Fe: m/z 56, 57, as Ga m/z 69; nM range</td>
<td>Only RT comparison with standards</td>
<td>Limited effects (e.g., coelution in void), co-elution with substances causing argon clusters</td>
<td>S</td>
<td>Routine method for common speciation analysis. Can quantify all element species including unknowns. Multi-element capabilities, SS-IDA and SUS-IDA.</td>
<td>Does not give direct speciation information. Only applicable for major non-interfered isotopes. Limited use for organic solvents</td>
<td></td>
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<tr>
<td>HPLC</td>
<td>DRC or CC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>As HPLC-ICP-MS but better LOD for interfered isotopes, e.g., Se: m/z 80, 6 ng/L</td>
<td>Only RT comparison with standards. Metal:S or metal:P ratios of molecules</td>
<td>Less than HPLC-ICP-MS. Elimination of interferences by specific reaction or collision Se (H&lt;sub&gt;2&lt;/sub&gt; to reduce Ar&lt;sup&gt;+&lt;/sup&gt;). S with Xe as collision gas</td>
<td>S</td>
<td>As HPLC-ICP-MS can be used for interfered isotope of metal(oids) (&lt;sup&gt;80&lt;/sup&gt;Se, &lt;sup&gt;54&lt;/sup&gt;Fe) or non-metals (&lt;sup&gt;32&lt;/sup&gt;S, &lt;sup&gt;31&lt;/sup&gt;P) direct determination of metal: S or P ratio in macromolecules.</td>
<td>Elimination only for specific isobaric interference, no direct knowledge of molecular clusters</td>
<td>As species in biological extracts, Cr (III/VI)</td>
</tr>
<tr>
<td>HPLC</td>
<td>HR-ICP-MS</td>
<td>fg-level DRC or CC ICP-MS in high HR mode, low HR for non-interfered isotopes</td>
<td>Only RT comparison with standards</td>
<td>Less than DRC/CC ICP-MS.</td>
<td>S</td>
<td>As HPLC-DRC/CC-ICP-MS. Knowledge of particular interference</td>
<td>Expensive technique</td>
<td>P, S speciation in macromolecules</td>
</tr>
<tr>
<td>Micro/nano HPLC</td>
<td>ICP-MS</td>
<td>150–200 fg Se</td>
<td>Only RT comparison with standards</td>
<td>No matrix effect due to low flow rates</td>
<td>S</td>
<td>Small sample volume. Use of 100% organic volatile solvents. High degree of ionization</td>
<td>Expensive Micro-HPLC pumps and columns. High LOD due to low sample volume</td>
<td>Cell extract. Protein digest from GE</td>
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<table>
<thead>
<tr>
<th>Molecular specificity</th>
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<th>Major advantages</th>
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<th>Major application</th>
<th>Ref.</th>
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<tr>
<td>CE</td>
<td>ICP-MS</td>
<td>fg-level</td>
<td>Only migration time comparison to standards with internal standardization</td>
<td>Significant matrix effect on migration time</td>
<td>S</td>
<td>As CE-ICP-MS. High species resolution. Gentle separation without interaction of stationary phase</td>
<td>Need to use internal standards for migration-time corrections. Low relative LODs</td>
<td>Seleno-amino acids MT</td>
<td>[14]</td>
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<tr>
<td>GC</td>
<td>ICP-MS</td>
<td>Superior LODs, e.g., ng S/g</td>
<td>Only RT comparison with standards</td>
<td>Matrix effect only at derivatization stage</td>
<td>NL, D</td>
<td>100% sample transport. High species resolution. Matrix-free detection</td>
<td>Limited applicability. Loss of speciation information due to the need to derivatize the species to form volatile species</td>
<td>Halogens, sulfur, methyl-Hg. Organotins. Both by SS-IDA</td>
<td>[11,24, 29,30]</td>
</tr>
<tr>
<td>GC</td>
<td>MC-ICP-MS</td>
<td>Only RT comparison with standards</td>
<td>As GC-ICP-MS</td>
<td>NL, D</td>
<td>As GC-ICP-MS, species-specific isotope ratios</td>
<td>Expensive instruments. Isotope fraction during chromatography</td>
<td>Pb, S in standard. Sb in landfill gas</td>
<td>[25–27]</td>
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<tr>
<td>GC</td>
<td>EI-MS</td>
<td>Species-specific LOD</td>
<td>Due to fragmentation pattern</td>
<td>Co-eluting organics can influence fragmentation</td>
<td>NL, D</td>
<td>Molecular information</td>
<td>Non-element specific. High LOD</td>
<td>Organotins, organoarsines</td>
<td>[32]</td>
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<tr>
<td>GC</td>
<td>Tunable plasma</td>
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<td>[43]</td>
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<tr>
<td>GE-LA</td>
<td>ICP-MS</td>
<td>Depending on blank level of gels 400 mg/kg Zn 0.6</td>
<td>Separates molecules of a large mass range</td>
<td>S</td>
<td>Quantification of macro-molecules</td>
<td>Tedious off-line method</td>
<td>Se-proteins</td>
<td>[17–21]</td>
<td></td>
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<tr>
<td>HPLC</td>
<td>ES-MS</td>
<td>Species specific</td>
<td>Due to M–H*, adduct and fragment formation</td>
<td>Fragmentation and adduct formation is severely matrix-dependent</td>
<td>S</td>
<td>Species-specific information</td>
<td>No elemental information</td>
<td>As species in biota</td>
<td>[33]</td>
</tr>
<tr>
<td>Method</td>
<td>MS/ICP-MS Quantification using ICP-MS (same LODs)</td>
<td>Species-specific M—H* adducts, fragments and element-specific</td>
<td>Limited matrix effect for quantification and identification</td>
<td>Quantitative element species information and species identification of unknowns possible</td>
<td>Compromised mobile phase for both ICP-MS and ES-MS</td>
<td>As–S species</td>
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<tr>
<td>HPLC</td>
<td>ES-IT-MS or ES-MS-MS</td>
<td>Species-specific ng/mL</td>
<td>Due to M—H* adducts, fragments and their daughter ions</td>
<td>Severe matrix effects by mobile phase</td>
<td>Often used off-line, needs clean up</td>
<td>As species in biota Se in yeast</td>
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<tr>
<td>Flow injection</td>
<td>ES-qTOF-MS or FT-ICR-MS</td>
<td>Due to M—H* and absolute mass/charge ratio</td>
<td>Severe matrix effects</td>
<td>Absolute mass determination result in knowledge of elemental composition</td>
<td>Needs isolated species in fraction, not useful for quantification</td>
<td>Organoarsenicals</td>
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<tr>
<td>Off line with GE</td>
<td>MALDI-TOF-MS</td>
<td>Molecular mass</td>
<td>No significant matrix effect</td>
<td>Only molecular peaks</td>
<td>Needs large volumes and high concentration in a cleaned-up sample</td>
<td>Labile As species during reaction</td>
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<td>^H NMR</td>
<td></td>
<td>Species-specific Proton interactions molecular mass</td>
<td>Needs purification</td>
<td>S, NL,</td>
<td>S, NI,</td>
<td>Se–S species</td>
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<tr>
<td>^37Se NMR</td>
<td>&gt;200 nM Se</td>
<td>Element-specific and its electronic near environment</td>
<td>No matrix effects for identification but severe for LOD or quantification</td>
<td>SS, NL,</td>
<td>Direct method for speciation analysis in solids, such as biota, soil or gel spots of 2D-GE for metalloproteins</td>
<td>Se–S species</td>
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<tr>
<td>^27Al NMR</td>
<td>0.1% Al</td>
<td>Depending on element and matrix 10 mg/kg for As in biota</td>
<td></td>
<td></td>
<td>Only the direct binding partners can be determined, not the entire molecule. Identification only as good as the standards</td>
<td>Al species in soil As speciation in biota. Organotin in sediment. Zinc in liver</td>
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<tr>
<td>XANES/EXAFS</td>
<td>Depending on element and matrix 10 mg/kg for As in biota</td>
<td>Element-specific and its electronic near environment (oxidation stage and bond length to ligands)</td>
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^aSS: Direct method for solids; S: Actual species is analyzed; D: Species needs to be changed by derivatization; NI: Non-invasive; NL: No direct methods for liquids.

^bDRC: Dynamic reaction cell; CC: Collision cell.

^cSS-IDA: Species-specific isotope-dilution analysis; SUS-IDA: Species-unspecific isotope-dilution analysis.
width of arsenite is 20 s, while the peak width of the late-eluting arsenosugar is 250 s. The LOD of arsenite is about 0.01 mg As/kg, while the LOD of the arsenosugar is about 0.1 mg As/kg.

However, co-eluting compounds (especially near the void volume) may alter the sensitivity of ICP-MS. This is not necessarily because of the formation of isobaric interferences but mainly due to a change in the plasma chemistry that alters the response of ICP-MS. Hence, co-eluting species cannot be quantified by species-independent calibration. This alteration of the sensitivity of ICP-MS during a chromatographic run can easily be monitored by adding a continuous internal standard (post-column), which can be detected simultaneously (see Fig. 1).

ICP-MS has traditionally been used for detection of metals and metalloids. Recently, a series of applications showed convincingly that non-metals, such as phosphorous, sulfur and silicon, can also be determined with reasonable LOD using ICP-MS as detector. This has mainly been possible using collision and reaction cells or high-resolution ICP-MS, which either decreases the background signal caused by isobaric molecular cluster interferences or avoids the spectral interference by separating the analyte signal from that of the molecular ion. Elements, such as phosphorous and sulfur (especially in biological systems), can be detected at a μg/L range.

2.1. High-performance liquid chromatography (HPLC-ICP-MS)

HPLC is almost routinely coupled to ICP-MS, as no sophisticated interface is necessary. It is widely used, so that ICP-MS has almost become a black box or routine detector for sophisticated chromatography and/or pretreatment. For example, it has recently been shown that, with sophisticated pre-treatment followed by chromatographic separation, enantiomers could be identified separately and quantified [3].

This development is enviable, but not without danger. For a long time, the common practice was that HPLC-ICP-MS was used to identify an element species by only the retention time. In such cases, species have been misidentified because unknown species of the monitored element can co-elute, so that even sample spiking would not rule out misidentifications. An example, illustrated in Fig. 2, shows that the recently identified dimethylarsinoyl acetic acid (DMAA) can easily been mistaken for a common arsenic metabolite, methylarsonic acid (MA(V)) [4]. Even the use of two-dimensional separation or pH variation of the mobile phase, which gains information about the molecular species, cannot necessarily prevent the risk of misidentification. Only lately has the use of molecular MS delivered unequivocal evidence of the occurrence of DMAA in the urine of sheep exposed to arsenic [4].

As mentioned above, LODs of element species are routinely in the sub-μg/L range if the elements do not suffer from isobaric interference, such as \(^{56}\text{Fe}^+\) does from \(^{40}\text{Ar}^{16}\text{O}^+\). The use of the dynamic reaction cell (DRC) or the collision cell (CC) has been beneficial for lowering LODs, not only for total element determination, but also for element-speciation analysis. For example, the most abundant selenium isotope, \(^{80}\text{Se}\), can be detected almost interference-free at a level of 6 ng/L, when methane has been used as a reaction gas [5]. Other notoriously interfered elements, such as sulfur (\(^{32}\text{S}, \(^{34}\text{S}\)) or

![Figure 1](http://www.elsevier.com/locate/trac)
phosphorous $^{31}$P), become accessible by using xenon as a collision gas [6] or oxygen as a reaction gas [7].

High-resolution sector-field ICP-MS makes use of mass resolution to detect molecular or isobaric interference. The technique has long been used for the determination of total element concentration, but only recently has its potential for speciation of non-metals been realized. A good example is the determination of several organophosphorous species in blood plasma and the determination of PDMS breakdown products and silanol by Carter et al. [8]. They achieved LODs in the lower $\mu$g/L range.

Lehmann and co-workers [9] have utilized HR-ICP-MS to detect sulfur interference-free and could identify the saturation of gadolinium-tagged macromolecules and peptides by their Gd/S ratio.

Another classical example is the use of HR-ICP-MS for the determination of iron. Harrington et al. [10] used a high-resolution ICP-MS to separate the ArO$^+$ background form their Fe$^+$, in order to perform iron speciation of biomolecules on the major iron isotope, $m/z$ 56.

An interesting alternative approach to detect very sensitive iron complexes has been reported by Moberg et al. [11]. Since they had no access to HR-ICP-MS or to a DRC/CC-ICP-MS, they derivatized the different iron species (siderophores) to overcome the iron interference. Siderophores are biomolecules that are excreted by plants and microorganisms to bind trivalent iron very strongly and make iron bioavailable. These researchers showed elegantly that a simple reduction of Fe(III) to Fe(II) in the presence of Ga(III) displaces the iron by gallium in various siderophores. The LODs using a quadrupole ICP-MS of four different siderophores in real soil-porewater samples have been reported to be in the nanomolar range, due to the almost background-free detection on $m/z$ 69 for $^{69}$Ga$^+$.

New exciting developments have been shown in the coupling of micro-HPLC or nano-HPLC to ICP-MS. These methods require very small sample volumes (5–500 nL) and, when used in combination with a total consumption nebulizer, a decrease of absolute LODs could be achieved. For example, Schaumlöfler and co-workers [12] demonstrated that the LODs of selenopeptides in tryptic digests of an isolated protein fraction are around 150–200 fg selenium. The use of micro-HPLC made it possible to use 100% organic solvents, such as acetonitrile, without cooling the spray chamber or using oxygen.

![Figure 2. Risk of misidentification if only HPLC-ICP-MS is used for species identification. Chromatograms of a standard solution containing the main arsenic metabolites [As(III), DMA(V), MA(V) and As(V)] and a urine sample measured on $m/z$ 75 using ICP-MS and simultaneously on-line recording of mass spectrum using ESI-MS of peak 3 (dimethylarsinoyl acetic acid). (Modified from [4].)](http://www.elsevier.com/locate/trac)
This has also been shown in a recent study, where a gradient of acetonitrile or methanol up to 100% has been used for the separation of five cobalamin species without any plasma variability [13]. Nano-HPLC has a comparable sample volume to CE, but does not suffer from the extent of matrix effects experienced using CE with real samples [14]. Both separation systems are highly valuable for future applications, especially in biosciences. Though some technical problems of interfacing these two instruments with the ICP-MS have been solved, future studies will show how robust these techniques are in routine bioanalysis.

2.2. Electrophoresis (CE-ICP-MS and GE-LA-ICP-MS)
Electrophoretic separation, dedicated to the separation of charged molecules, is a gentle way to separate compounds, in particular those that are labile. CE and recently capillary electrophromatography (a combination of nano-HPLC and CE) have increased in popularity due to the demands in the bioanalytical field (e.g., to determine metabolites in cytosols of cell cultures). In this case, only 10-nL samples are usually available, because not more than 10 million cells are used in cell cultures.

The coupling of CE to ICP-MS is not as straightforward as that of HPLC to ICP-MS. This is because a low dead-volume interface is needed, as in micro-HPLC or nano-HPLC, with typical flow rate <0.5 μL/min [15]. However, a commercial interface for CE-ICP-MS is now available [16].

CE has a theoretically high resolving power and can separate ions of different charges, but, so far, CE has not made the breakthrough in element-speciation studies because of several limitations. The absolute LODs achievable are in the high femtogram range, but the concentration of metals and metalloids in real samples are often too low for these LODs due to the low injection volume. Preconcentration of the sample is not an option, since CE suffers from severe matrix interferences that alter the migration time of the species. This latter effect can be cancelled out only by using multiple internal standards as time markers from which the migration time can be calculated [14].

With the major focus of elemental speciation on the determination of macromolecules (metallo-enzymes and proteins), it becomes inevitable that classical bioanalytical separation techniques, such as CE, are being explored. To date, only a few studies have been published on the combination of GE with solid sampling ICP-MS [17–21]. The combination is off-line.

Laser ablation (LA)-ICP-MS is used as a solid sampling technique to map the gels for their metal and non-metal content, either with line scans or with raster scans. Different strategies for quantification have been used. Interesting is the quantification of proteins and their phosphorylation by quantifying P and S concentrations in gel spots of 2D gels with a solution-based calibration, by using a standard solution introduced as a dry aerosol [20]. LODs seem to be determined by the blank level of the gels when a Nd:YAG laser and a sector field ICP-MS are used; zinc could be detected only above 400 μg/g, while aluminum could be detected at 0.6 μg/g. It is astonishing that the authors achieved a similar detection level for phosphorous using medium resolution of m/Δm of 4000 in order to separate the background signal caused by 31P+, 14NOH+ from interference-free detection of 31P+.

Another example is the detection of interference-free selenium of selenoprotein after a high-resolution separation using 2D GE and LA-ICP-MS detection [21]. The authors used carbon monoxide as a reaction gas in the DRC to reduce the Ar2+ background on the major selenium species m/z 80.

The off-line coupling of GE with LA-ICP-MS is a real advance in the determination of metalloproteins in comparison to the traditional non-elemental selective staining methods or expensive element-specific techniques, such as synchrotron radiation XRF [22].

There is still a lack of knowledge about the stability of metalloproteins using GE. To tackle this question, new quantification strategies have to be developed in order to assess accurately the extent of possible transformation reactions during the separation step.

2.3. Gas chromatography (GC-ICP-MS)
CE-ICP-MS and GC-ICP-MS share the same advantage – the sample transport into the ICP-MS is nearly 100%. Since capillary GC also has a high resolving power, GC-ICP-MS is an attractive speciation method for volatile and thermally stable compounds.

However, volatile metal(loid) species are rare and often not thermally very stable (e.g., methylated arsines, stibines and stannanes have shown that they can decompose or adsorb irreversibly in the GC column [23]).

But, using DRC/CC, it has become possible to monitor additional elements often found in volatile organic species, such as sulfur or halides. Bouyssiere et al. [24] have shown that direct sulfur speciation in petroleum products is achievable at the ng/g range. They reduced the O2− background in the CC with helium, which made the major sulfur isotope, 32S, accessible for quantification.

Prange and co-workers [1] demonstrated nicely that the addition of helium in the CC and the optimization of the bias setting between octopole and quadrupole is necessary to create the largest kinetic energy discrimination for the interfering molecular ions and the elemental ions. They managed to find a compromise CC condition using helium (2.5 mL/min) for the detection of 31P, 32S, and the halogens 35Cl, 79Br and 127I. Hence, they utilized the element ratios and the retention time for the identification of organopesticides.

LODs at the lower μg/L level have been achieved by adding helium to the injector gas. This is clearly an
achievement, which will be noticed by environmental analytical chemists, and more applications in the determination of volatile species are expected.

Other applications of GC-ICP-MS have focused on precise species-specific isotope ratios in volatile species, such as SF$_6$ [25], PbEt$_4$ [26] and SbMe$_3$ [27]. The emphasis of these studies is not on the achievable LODs but more on the precision and the accuracy of the species-specific isotope ratios. Wehmeier et al. [27] have investigated how the isotope ratio of antimony (m/z 123/121) varied from point to point during the elution of the analyte species, here trimethyl stibine. An isotope-ratio shift of antimony in the eluting trimethyl stibine has been detected, due to the high precision of 0.02–0.08% using GC-MC-ICP-MS. However, this point-to-point fractionation shift (as seen in Fig. 3) is not observed by the isotope ratio of cadmium (m/z 111/110) that is measured simultaneously. However, it is remarkable that the heavier $^{121}$SbMe$_3$ elutes ahead of the $^{123}$SbMe$_3$ [27]. But, this finding is consistent with an observation made by Guenther-Leopold et al. [28], who observed a preferential transmission of the heavier isotope of xenon and krypton, without any chromatography. The authors recognized that this unexpected reverse fractionation effect only takes place in a dry plasma. Uranium isotope-ratio measurements using transient signals from solution introduced via flow injection or HPLC did show a fractionation, but this time the lighter isotope was preferentially transmitted. This again contrasts with the findings for SbMe$_3$, which was measured also under wet plasma conditions. Although it is assumed that the plasma or the interface can change the mass bias when a transient signal is recorded, isotope fractionation during chromatography cannot be excluded. More studies concerning the mass-bias behavior in a multi-collector ICP-MS in dry and wet plasma conditions are necessary to answer the question of whether chromatographic fractionation does occur to a degree that can be measured.

GC-ICP-MS has been used for not only volatile species, but more often applications in which organometallic compounds have been extracted and derivatized to volatile species (e.g., organotins have at least one or two free valences and do not readily occur as volatile compounds in environmental and biological samples). Tributyl tin (TBT) binds in water most likely to OH$^-$ or Cl$^-$. However, butyl tin compounds, alkyl lead or methyl mercury can be derivatized to fully alkylated species. Sodium tetraethylborate or tetrapropylborate have been used routinely, where ethyl or propyl replaces chloride or hydroxide quantitatively. Excellent LODs in the sub-ng/L range have been reported using GC-ICP-MS, due to the 100% sample introduction and sharp narrow peaks.

The LODs can be further decreased by using a combination of solid-phase microextraction (SPME) and multi-capillary GC [29]. Due to the matrix problems associated with the derivatization step in real samples, species-specific isotope-dilution analysis (SS-IDA) has been developed to a high sophistication. Rodriguez-Gonzalez et al. [30] have demonstrated successfully that a triple spike for organotin compounds can account for all transformation reactions taking place during the sample-preparation steps. It has been recognized, that the spike does not react in the same way as the analyte in the sample. Recoveries of more than 100% point to a stronger binding of the TBT spike to the matrix than the original TBT in the sample. However, the necessary condition for SS-IDA is that the spike of the isotopically labeled species has to be equilibrated with the sample. Whether or not this has taken place is difficult to assess, since the analyte might bind to different ligands from the spiked species, which is quite likely when biological samples or tissues are spiked. The original organo-metallic compound can bind to various enzymes, proteins or peptides, which were perhaps abundant in vivo but not available at the time of the spiking. Fundamentally, a biological tissue can never reach an equilibrium. Ultimately, the extraction/derivatization efficiency may not necessarily be the same for the spike and the analyte [30]. Methods capable of providing information about the molecular occurrence of the element directly in the sample are expected to shed more light on the observed effect.

3. Molecular MS

Electron impact ionization is mainly used in combination with GC, while a number of different ionization methods, such as ES, MALDI, atmospheric pressure chemical ionization (APCI) and, more recently, atmospheric
pressure photoionization (APPI) [31], have been used for liquid and solid samples.

3.1. GC-MS
GC-MS methods will not be discussed in great detail, as no real advances have been made in recent years in the molecule-specific detection of volatile compounds. However, it should not be forgotten that highly sensitive GC-MS methods are available to gain molecular information. This has been demonstrated in a recent study, which convincingly showed the occurrence of new volatile arsenic species in geothermal vents using GC-MS [32].

3.2. ES-MS
The advances in ES-MS have had an enormous impact on speciation studies, in particular on arsenic and selenium speciation due to their rich organic chemistry. The methods could be used to identify unknown species, even without the presence of a standard. However, ES-MS has some serious limitations, which prevent this technique from being superior to others:

- First, it lacks element specificity, although it has been demonstrated that arsenosugars can be ionized to As⁺ (m/z 75) when oxygen-free nitrogen is used as a drying gas. However, in this relevant study, only a few percent of the protonated molecular ion was fragmented to 75As⁺ [33].

- Second, some species are difficult to ionize using ES ionization (e.g., dimethylarsenous acid [DMA(III)]) [34]. Although the ionization efficiency of some species can be comparable to ICP-MS (in dilute solutions), the LODs for other species can be two-to-three orders of magnitude higher than those achieved by ICP-MS. This species-specific ionization by ES-MS is illustrated in Fig. 4, which shows a mixture of two arsenic species (DMA(V) and DMAS) in a hair extract. The m/z 75 trace of the ICP-MS shows directly the concentration ratio due to the species-independent response on the ICP-MS, while DMA(V) seems to be easy to ionize in comparison to DMAS on the m/z 75 trace from the ES-MS. Additionally, ES-MS indicates a third peak, which resulted from an organic compound eluting in the void, indicating that m/z 75 is not arsenic-specific.

- Third, it is fully established that the ionization efficiency is suppressed by co-existing matrix [35]. If chromatographic separation was employed to clean up the sample prior to analysis, this matrix may be the buffer from the mobile phase and/or the co-eluting sample matrix. This detrimental effect makes a reliable quantification by external calibration using a diluted standard inaccurate and a species-independent calibration impossible. Hence, in most cases, ES-MS is used for identification purposes only. However, the molecule specificity of ES-MS-MS or ES-ion trap-MS can be utilized in particular for the direct measurements of crude extracts when selected reaction monitoring is employed. This method has elegantly been demonstrated by Francesconi and Pergantis [36]. They identified and quantified a minor relatively unstable arsenic metabolite at the μg/L level directly in a crude extract without any clean up.

Other ES-MS instruments are designed to determine accurate mass. For example, due to the mass deficiency of arsenic, As has an accurate mass of 74.92, so a crude solution can be scanned for mass-deficient fragments. So far, no calculation routine has been reported. However, if the accurate mass is determined, it is possible to identify the entire elemental composition.

In a recent study, the data obtained by ES-quadrupole time-of-flight (qToF)-MS was based on information already obtained and accurate enough to confirm the molecular formula of an unknown product (153,948 Da) as C₂H₂-OSAs [153,950 Da, i.e., dimethylarsinothioic acid (DMAS)] [34]. The mass error of the ES-qToF-MS results was 10 parts per million (ppm), which is in the range of what can be expected.

When analyzing arsenic compounds in a complex matrix, Pickford et al. [37] achieved a mass accuracy of 2 ppm on a qToF instrument (Micromass), but implied that this level of accuracy was not routinely achieved.

Molecular identification of an unknown compound based solely on mass accuracy will in most cases require a mass accuracy of parts per billion (<10⁻⁶). Such a high mass accuracy can be achieved on a FT-ICR-MS (Fourier transform-Ion Cyclotron Resonance-MS) instrument, as was shown by Pickford et al. (who achieved a mass error of 0.22 ppm) [37].
3.3. MALDI-MS

MALDI-MS is a gentle ionization technique, which cannot be coupled on-line with a chromatographic technique. Although an automated, fractionation/sample-preparation set-up has been tested for organic compounds, so far no study on elemental speciation has been reported. In contrast to ES, MALDI does not suffer from matrix-effected ionization suppression and is often more efficient due to the smaller number of fragments and adducts formed [38]. This method has mostly been used for large biomolecules, such as polypeptides. Also LODs down to 100 picomolar (pM) have been reported with conventional MALDI-TOF-MS [39]. The few examples published in the field of elemental speciation by MALDI-TOF-MS have been on the identification of selenium-containing polypeptides in tryptic digests from purified proteins [40]. But non-peptide selenium species from selenized yeast have also been reported [41].

4. Dual detection systems

Due to the nature of element species occurring in trace amounts in either biological solid material or fluids, which contain an enormous number of different organic compounds – often in much higher concentrations than the trace amounts of element species – is it desirable to use an element-specific detector to identify the “needle in the haystack”? The identification of the element in the “organic cocktail” certainly does not characterize the entire molecule, so additional molecular information is required. Ideally, an element-specific quantification method should be coupled to a molecule-specific method to gain reliable identification and quantification of element species in a sample (Fig. 5).

4.1. Off-line systems

On-line systems, such as HPLC-ICP-MS, have often been used to identify fractions containing element species of interest. The fractions were then, in most cases, subjected to flow-injection ES-MS or ES-MS/MS to obtain the molecular structure information. The advantage of these systems is that compromises about the separation are not necessary and the fraction can be further manipulated in order to satisfy the requirements of the ES systems. For example, in detecting selenosugars in human urine, it was necessary to clean up the fraction by lyophilization and size-exclusion chromatography in order to preconcentrate the sample and reduce the matrix [42]. The problem with an off-line set-up is that the technique is time consuming and cannot easily be automated. Furthermore, there is a greater risk of contamination and species transformation of labile species due to the different sample-manipulation steps.

4.2. On-line systems

Ideally, both the elemental and the molecular information can be retrieved from one chromatographic run online. Limited sample preparation is involved and the results are better traceable. Two different approaches have been realized in recent years: the development of tunable plasmas; and, the simultaneous use of ICP-MS and ES-MS as a dual detection system for one chromatographic system. ICP-MS with a tunable plasma that can be operated in either elemental or molecular mode has been reported in the literature [43,44]. Also radiofrequency glow-discharge helium plasma MS systems have been tested as detectors for GC and HPLC [45]. Superb LODs of sub-pg of alkyltins have been determined [46]. These plasmas can switch from atomic to molecular MS within microseconds. Basically, a multi-dimensional chromatogram can be generated, which can give element-specific, as well as molecular, information. However, glow-discharge plasmas as tunable plasmas are in the development stage and so far not commercially available.

An HPLC-ES-MS/ICP-MS system can easily be realized by combining commercially available instruments. We have coupled a fully-automated HPLC directly via a micro-splitter to ICP-MS and ES-MS. The entire system is electronically connected and runs fully automatically. ICP-MS is used for quantification, while ES-MS is used for identification purposes only. Advantages are that it is not time consuming and it is suited for labile species. Once the sample is generated, it can be held under nitrogen at 4°C and immediately subjected to the analysis.

In addition, an HPLC-ICP-MS/ES-MS system is particularly suited to identifying species transformation during chromatography. Raab et al. [47] have shown that
arsenic-glutathione complexes are unstable and can disintegrate during a routine chromatographic method (PRP X-100 strong anion-exchange chromatography). This method has extensively been used by many groups for arsenic-speciation studies. Only the use of organic solvents and a reverse phase chromatography at 6°C guaranteed that dimethylglutathionyl arsine (Me$_2$AsGS) could elute unchanged, like MeAs(GS)$_2$ and As(GS)$_3$. Furthermore, the combination of parallel, on-line use of ICP-MS and ES-MS has made it possible to identify a series of novel arsenic metabolites in plants and animals. For example, not only different arsenic-phytochelatin complexes (AsPC$_3$, GSAsPC$_2$ and As(PC$_2$)$_2$) were identified and quantified, but, in the same run, the amount of oxidized glutathione and free phytochelatins were determined [48].

However, the major limitation is quite often that the LODs of the ES-MS system, in particular for species that show a low ionizability, such as DMAS or DMA(III) [34], are significantly higher than those of the ICP-MS. Without sample pre-concentration and/or clean-up procedures, satisfactory molecular identification is not possible due to the enormous matrix effect of the ionization process in ES-MS. The high concentration of salt from the eluent, in particular when so-called physiologically identical conditions, such as a TRIS buffer used for gentle separation, suppresses the ionization that eventually leads to very high LODs. Furthermore, if an on-line system is used to analyze for a number of element species, whose natures are unknown, a scanning detection mode, over the full mass spectrum, has to be used for the ES-MS. Consequently, this increases the LODs further. However, if a structure is predicted, the major fragments of the anticipated species can be monitored in selected ion monitoring (SIM) mode, and the LODs can be improved by a factor of 10 on switching from scanning to SIM. By altering the fragmentor voltage of the ES-MS, mainly protonated molecular ions (100 V), and fragment ions (200, 400 V) can be observed. However, often MS/MS experiments are necessary for species identification. This is possible in an off-line system with fraction collection after multiple separations [49].

However, the analyst should be aware of common problems that limit the detection capabilities of this “dream team in life science”, as Wind and Lehmann [50] recently named elemental and molecular MS.

Chromatography and sample preparation are probably the weakest links in a coupled system. It is a fact that the chromatographic recovery (defined as the sum of species of an element eluting from a column in relation to the total amount of the element in the injected sample) is often not quantitative. Hence, a large percentage of species is therefore unaccounted for, which has the consequence that the speciation must be considered thus far incomplete.

These hidden, non-eluting species have been found to a certain extent in the certified reference materials: arsenic in DOLT-2, DORM-2 and TORT-2 [51]. Interestingly, the arsenic species in an extract of a CRM hair sample (GBW09101) has shown nearly quantitative chromatographic recovery, while the same analytical method reveals only less than 50% when applied to sheep’s wool [52–54].

A low chromatographic recovery has also been found for arsenic species in urine from sheep feeding solely on seaweed containing high concentrations of arsenosugars [55,56].

Routine chromatographic methods, largely used in arsenic-speciation studies, may not reveal many compounds, since they basically do not elute from the separation column. Not only should optimization of the extraction efficiency be the focus of the investigations, it should also be good laboratory practice to identify the chromatographic recovery in order to report the hidden species. Thus, it was possible using this dual ES-MS and ICP-MS system to identify a new class of arsenic metabolites – organo-thioarsenicals — by just changing chromatographic conditions from those used routinely [56]. These organo-thioarsenicals “stick” to certain anion-exchange columns, and it seems that the interaction is not due to the ion-exchange processes but rather to strong interactions with the polymer backbone [57].

In order to identify the nature of the new compounds, ICP-MS data revealed that arsenic and sulfur co-elute, while the ES-MS gave on-line fragments that would suggest the elution of Me$_2$As(S)CH$_2$COOH – a new class of arsenic compounds. However, only the analysis using off-line ES-IT-MS confirmed the nature of the different fragments by using MS$^2$ and MS$^3$ and the existence of this new metabolite in sheep’s urine.

As mentioned above, using off-line systems has the danger that labile compounds transform. It has been observed that, in collecting a fraction from the chromatographic system, the fraction had to be cooled in dry ice, otherwise the compounds would have been oxidized to an earlier observed arsenic metabolite, DMAA [4].

The picture of the occurrence of element species in biological and heterogeneous samples has been shaped by the information that we have retrieved from the different analytical systems, although it has been acknowledged that mainly the most stable and abundant species have been identified so far. Extractions may intrinsically change the chemical environment of the compounds (i.e. the species are often stabilized by pH, ionic strength or the concentration of particular ligands) as well as redox regime. The sample-preparation steps have to be revisited and assessed by other alternative techniques and changed if necessary, so that they satisfy the criteria that the species integrity is guaranteed and can be applied to elemental and molecular MS.
None of the methods mentioned so far can measure the species \textit{in situ} and in real time. In the remainder of this article, I discuss a few emerging techniques and their applicability to elemental speciation.

5. XAS

XAS comprises the two distinct methods XANES (X-ray near edge spectroscopy) and EXAFS (extended X-ray atomic absorption fine structure). These techniques cannot determine the composition of an entire compound, but they reveal information about the near electronic environment of an element in a heterogeneous non-crystalline mixture. These techniques are only two of many techniques, such as XPS (X-ray photoelectron spectroscopy) and PIXE (proton-induced X-ray emission), which can be used directly on the undisturbed sample. But the recent development in optics and increased accessibility have shown the potential of XANES/EXAFS for speciation analysis.

Although the information from XAS on species distribution is somewhat restricted, XAS has been a tool in material sciences, especially catalysis, for many years, but has only recently shown its potential in environmental and biosciences due to the installation of “ultra-dilute beamline stations” at synchrotron radiation sources for the measurement of ultra-dilute samples.

XANES can mainly reveal the oxidation state of the element and its binding atoms, whereas the bond length to the nearest atoms and its coordination number is easier to extract from EXAFS data. Consequently, the remainder of the molecule cannot be characterized.

Since the absorption edge, although species-specific, does not have a high discrimination potential, XANES and EXAFS can provide information only about major species in a mixture of species if the species are known. A problem occurs if there are compounds in the sample that are not anticipated. Also, if the coordinates of the element are not known, the correct identification of species in a mixture is challenging. Quantification is even more difficult, since the species distribution can be determined only by modeling the convolved absorption curves with absorption data of pure standard compounds.

However, the absolute advantage of both techniques is that they do not require any sample-preparation steps, i.e. element species can be measured \textit{in situ} in the biological samples, hence the chemical environment of the element is not changed. For example, it has been possible to identify major arsenic species in arsenic-tolerant earthworms as trivalent arsenic, which binds to reduced sulfur, as in glutathione (see Fig. 6) and as pentavalent arsenic, which binds to four oxygen atoms, as in arsenate [58]. The analysis of the methanol water extract, from the same samples, by HPLC-ICP-MS and ES-MS revealed that arsenic occurred in the majority as arsenate and unexpectedly arsenite in addition to minor amounts of arsenobetaine. Hence, the HPLC-ICP-MS and ES-MS data indicated that arsenic was present mainly as unbound inorganic arsenic. Only the \textit{in situ} techniques revealed that this was not the case. Later, it was shown that arsenic-glutathione compounds, which might have been in the extract, are unstable and decompose during the chromatographic separation process [47].

The LOD of the species identification by XANES and EXAFS depends on the flux of the synchrotron radiation, the detector, the matrix and the element. For example, a signal for arsenic in a biological sample as low as 10 mg/kg can be detected at the Daresbury synchrotron radiation source, although the sample has to be measured for more than 15 hours to collect data, which allows reliable EXAFS modeling [59]. New optical arrangements, detectors and higher fluxes may in the near future increase the detectability of element species in biota by a factor of 10. Hence, these techniques can also be applied to biological samples of background concentration and not only to those that...
accumulate high levels of the element (e.g., hyper-accumulator) [58].

First studies using control groups have been published [60, 61]. The hepatic zinc in trout, exposed to high levels of zinc, was shown to be in the same bound form as the zinc in the control group using XANES and EXAFS. Zinc, at concentrations of 13–22 mg/kg, binds to four sulfur atoms in the form of reduced sulfur, as in cysteine [60].

In another study, the LODs of less than 10 µg/g achieved for tin are promising. It would make it possible to determine the ratio of organotins and inorganic tin species in real samples, such as paint chips or sediments, without extraction and derivatization [61].

Furthermore, direct speciation can be performed in trace elements with spatial resolution of a few micrometers. µ-XANES has been used for the localization of tetravalent- and hexavalent-depleted uranium in small soil particles from Kosovo [62].

A new combined use of synchrotron-based X-ray fluorescence, diffraction and absorption has been successfully demonstrated by Manceau et al. [63] to identify five new zinc species in a clayed, acidic soil, comprising a concentration of 128 mg/kg with a spatial resolution in the micrometer scale.

6. Other methods

Raman and infrared spectroscopy have been applied to element-speciation analysis. Fourier-transform infrared (FT-IR) spectroscopy has been used for the indirect determination of selenium by its effect on Escherichia coli bacteria at a µg/L range [64]. The authors claim that FT-IR in combination with the bacteria could be used to determine the different selenium species in an aqueous sample, by analyzing the selenium effect of the bacteria. Perhaps this has the potential to use micro-organisms as amplifiers for specific element species to which they are exposed.

Raman spectroscopy has been used for the direct determination of arsenic species in iron hydrates in mine tailing ponds [65]. However, the concentration of arsenic was in the percentage range.

Nuclear magnetic resonance (NMR) spectroscopy has not often been used for metal-speciation analysis due to its high LODs and problems associated with impurities. In general, ¹H NMR and ¹³C NMR can be used to identify molecular details of the elemental species. Due to its lack of specificity to the element species, the analyte has to be pre-concentrated and separated from the matrix. This art has been mastered by natural-product chemists, who rely entirely on NMR and MS for their product identification. Analysts may be able to apply those schemes to the analysis of element species. Although NMR is not invasive and can identify the species in situ, only a few studies have demonstrated that the LODs achievable are low enough for real samples.

However, due to the absence of chromatographic separations, labile, short-lived compounds can be detected during a chemical reaction in a model solution, illustrated recently by the reaction of hydrogen sulfide with dimethylarsinic acid [DMA(V)] [34]. The short-lived and genotoxic trivalent arsenic analogue, DMA(III), has been recorded as an intermediate due to its chemical shift as a minor compound (see Fig. 7).

Furthermore, it has been demonstrated that ¹H NMR data of unsubstituted arsenosugars, generated when liver homogenates were incubated with arsenosugar-

![Figure 7. Time-dependent scanning of the methyl singlets, using 400 MHz ¹H NMR, of arsenic species. Monitoring of a simulated cytosol reaction, where DMA(V) reacts with H₂S to form the genotoxic intermediate DMA(III), Product B, and finally to dimethylarsinothioic acid (DMAS, Product A). (Modified from [34].)
containing seaweeds, can reveal the occurrence of two thio-arsenosugars anomers, which would have not been detected by molecular MS [66]. NMR can be used more elegantly when the element of interest can be studied directly by NMR. However, not all isotopes are NMR active and generate sharp NMR signals. But it has been shown that aluminum speciation in soil could utilize the signal of $^{27}$Al to determine the signals. But it has been shown that aluminum speciation isotopes are NMR active and generate sharp NMR interest can be studied directly by NMR. However, not all thio-arsenosugars anomers, which would have not been containing seaweeds, can reveal the occurrence of two 7. Concluding remarks

The field of elemental speciation has been widened in the last few years due to the developments in analytical methods that can determine the molecular form of elements in real samples. A characterization of element species in, for example, biological samples benefits from the use of a series of methods with complementary information about the species occurring. It becomes ever more important to have access to metal species that are difficult to extract, such as those bound non-covalently to peptides, polypeptides and proteins or lipids [48,70]. In particular, the development of the direct speciation methods, which have become sensitive enough for background samples will change our view of how traces of element species occur in biological and environmental samples. Spatial resolution of elemental speciation will be a major focus in future. Hence, the development of more sensitive $\mu$XANES and nanoHPLC systems or other methods using only small volumes and providing femtogram LODs will also advance the field, since the ultimate goal is to perform elemental speciation analysis in real time in the different compartments of single cells.

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